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Ultraviolet Radiation Inhibits Alloantigen Presentation by Epidermal Cells: Partial Reversal by the Soluble Epidermal Cell Product, Epidermal Cell-Derived Thymocyte-Activating Factor (ETAF)

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It has been postulated that ultraviolet radiation (UVR) alters antigen presentation by macrophages. This is thought to be due, in part, to inhibition of macrophage-derived interleukin 1 (IL-1), which is a hormone-like factor with immunoregulatory functions. Conventional stimulator cells for antigen presentation are macrophages; however, other cell types such as epidermal Langerhans cells are capable of antigen presentation. Keratinocytes also play a role in the immune system by providing a factor with IL-1-like activity, termed *E*pidermal cell-derived *T*hymocyte-*A*ctivating *F*actor (ETAF). The purpose of this study was to determine whether UVR affects alloantigen presentation by epidermal cells and if so, whether the UV-induced change is due to UVR alteration in ETAF activity. Epidermal cells from UV-treated BALB/c mice (UV-EC) or from non-UV-treated mice (EC) were x-irradiated and then cocultured for 5 days with allogeneic T cells from C57Bl/6 mice. UV-EC caused less T-cell stimulation than did EC from non-UV-treated animals. When chromatography purified fractions of ETAF were added to cultured UV-EC, partial restoration of T-cell stimulation was seen. These results suggest that this UV-induced defect in alloantigen presentation is due, in part, to decreased ETAF activity.

The mixed lymphocyte reaction involves the interaction of T cells (responder cells) with I-region determinants of allogeneic cells (stimulator cells). Macrophages [1–4], and, in particular, Ia-bearing macrophages, are considered to be the major stimulatory cells in a mixed lymphocyte reaction [5–7]. The mixed lymphocyte reaction has been used for studying the basic mechanisms of alloantigen recognition. It can be considered an in vitro correlate of the graft-vs.-host reaction [8] and allograft rejection [9], and is especially useful in selecting the best donor–recipient combination when several potential donors are available for organ transplantation. It has been demonstrated that apposition between macrophages and T cells is required for allogeneic T-cell stimulation [10], and recent studies suggest that hormone-like antigen nonspecific factors are required to augment the reactivity of responder T lymphocytes for optimal T-cell stimulation [11–14]. It appears that a helper T cell must receive two types of signals in order to respond to soluble antigen [12,15]. First, the T cell recognizes antigen in association with Ia antigens on the surface of the macrophage. The second signal is provided by a nonspecific helper factor produced by the macrophage [15]. It has been postulated that this soluble helper factor is interleukin-1 (IL-1) [16].

Lafferty and Woolnough have shown that ultraviolet radiation (UVR) can destroy the ability of cells to act as stimulators in the mixed lymphocyte reaction [12] and they have suggested that this defect was due to an alteration in the soluble second signal. More recently, Germain has demonstrated that UV-induced inhibition of antigen presentation (UVR given after antigen pulsing) could be restored with phorbol myristic acetate (PMA), which stimulates IL-1 production [17].

In addition to the conventional stimulator cells used for antigen presentation, namely macrophages, epidermal Langerhans cells, are also capable of antigen presentation [18–20]. Langerhans cells are the only epidermal cells capable of this function. Keratinocytes also play a role in the immune response by producing a factor that is biochemically and functionally similar to IL-1. This factor has been termed *E*pidermal cell-derived *T*hymocyte-*A*ctivating *F*actor (ETAF) [21]. In addition, ETAF is produced by the spontaneously transformed keratinocyte cell line Pam 212 [22].

In contrast to macrophages, which reside in tissues not normally exposed to UVR, Langerhans cells reside in the epidermis which is constantly exposed to UVR. Since we, as well as others, have shown that UVR can alter antigen presentation by murine epidermal cells [23,24], we sought to determine whether UVR would alter alloantigen presentation by epidermal cells and whether this was related to alteration in ETAF activity. The results of this study show that UVR can inhibit alloantigen presentation by epidermal cells and that this alteration is associated with decreased ETAF activity. In addition, supplementation by exogenous ETAF is capable of partially reversing the defect in antigen presentation.

MATERIALS AND METHODS

Mice

Specific-pathogen-free female mice of BALB/c AnN strain were supplied by the Animal Production Area of the Frederick Cancer Research Facility (Frederick, Maryland). The mice were 8–12 weeks old. C57Bl/6 mice were obtained from Jackson Laboratories (Bar Harbour, Maine) and were 12–16 weeks old when used. C3H/HeJ mice were obtained from Jackson Laboratories and were 8–12 weeks old.

UVR Treatment of Mice

A 2.5-kW xenon arc was used as the light source, coupled to UV interference filters (Corion Corp., Waltham, Massachusetts), of peak transmission at 270 nm as previously described [25]. An area of irradiation of 50–60 cm^2 was produced, allowing for the total dorsal irradiation of 3 mice simultaneously. The dose delivered was 175 J/m^2. Irradiance measurements were made using an International Light Radiometer

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Abbreviations:
αIa + C′: anti-Ia antiserum plus complement
EC: epidermal cells from non-UV-treated mice
ELR: epidermal lymphocyte reaction
ETAF: epidermal cell-derived thymocyte-activating factor
FCS: fetal calf serum
IL-1: interleukin 1
2-ME: 2-mercaptoethanol
NMS + C′: normal mouse serum plus complement
PHA: phytohemagglutinin
PMA: phorbol myristic acetate
UV-EC: epidermal cells from UV-treated mice
UVR: ultraviolet radiation

(model 700) coupled to a UVB detector (#PT171C) containing a WB320 interference filter with a cosine correcting diffuser. Nine measurements were taken across the field before and after each exposure. The average was used to calculate the dose. Additional measurements were made using an Optronics Spectroradiometer (Model 742, Optronics Laboratories, Orlando, Florida). Irradiances were in agreement to within 10%. The hair was removed from the back skin of the mice (approximately 10 cm^2) by plucking, immediately before irradiation. The mice were placed in a quartz-covered lucite container (11.5 cm diameter × 5 cm depth) which had air holes in the sides and 3 individual compartments. Control mice, epilated as above, were placed in an identical container kept in the dark for the same period of time as the UV irradiation. Both control and UV containers were set atop turntables rotating at 3 rpm. Exposure times were under 2 h. Immediately following treatment, mice were returned to their conventional cages. The wavelength of 270 nm was chosen since we wanted to avoid altering the morphology of Langerhans cells which occurs at doses greater than 100 J/m^2 [26]. In addition, narrow-band radiation of this energy caused no gross alteration in the epidermis and no inflammatory infiltrate was seen.

Culture Medium

RPMI 1640 containing 100 units/ml penicillin and 100 μg/ml streptomycin (Microbiological Associates, Walkersville, Maryland) supplemented with 10% heat-inactivated fetal calf serum (FCS, Microbiological Associates) 5×10^{-5} M 2-mercaptoethanol, and 2 mM glutamine (Grand Island Biological Company, Grand Island, New York) was used.

Epidermal Cell Suspension

Animals were sacrificed and back skin was obtained 24 h after UV irradiation. Epidermal cells were prepared by trypsinization of whole skin (only treated area or control area of back skin was used) as previously described [21]. The bulk of the epidermal cells are keratinocytes; the remaining cells are Langerhans cells (approximately 2–5%) and melanocytes (approximately 5%).

Responder T Cell Preparation

Spleens from female C57Bl/6 mice (12–16 weeks old) were disrupted with a syringe and forceps. A cell suspension was obtained and washed 3 times in RPMI 1640 (GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated FCS (GIBCO). In order to obtain a purified T-cell population depleted of Ia-bearing macrophages and B cells, the spleen cells were treated with a 1:10 dilution of polyspecific anti-Ia antiserum (A.TH anti A.TL) plus a 1:10 dilution of guinea pig complement (GIBCO) for 30 min at 37°C and then passed over a nylon wool column. Thus, in order to achieve T-cell stimulation in this system, epidermal cells would have to serve both as stimulator cells and as accessory cells. Nonadherent cells were then washed 3 times and incubated for 30 min at 37°C in a plastic Petri dish (Falcon, Oxnard, California). The nonadherent population contained more than 90% T cells as determined by anti-theta staining. The purified T-cell population was then washed and adjusted to 2×10^6 viable cells/ml.

Allogeneic Epidermal Cell Lymphocyte Reaction (ELR)

Epidermal cells (2×10^5, 1×10^5, or 0.5×10^5) from UV- or non-UV-treated BALB/c mice were treated with 2000 rads x-ray, then incubated with 2×10^5 C57Bl/6 T cells in round-bottom plates (Dynatech Laboratories Inc., Alexandria, Virginia). Wells with T cells alone were used to determine background counts. Cells were cultured for 1–14 days at 37°C with 5% CO_2 in humidified air. Sixteen hours before termination of the cultures, 0.5 μCi tritiated thymidine ([^{3}H]TdR 1.9 Ci/mm, New England Nuclear, Boston, Massachusetts) was added. Cells were collected on filter paper with the aid of a Mash II harvester (Microbiological Associates) and the ^{3}H activity on the filter discs was counted in a scintillation counter. The results are expressed as the mean cpm [^{3}H]TdR incorporation ± SE of triplicate cultures.

Assessment of ETAF Activity from UV- and Non-UV-Treated Animals

Epidermal cell suspensions were prepared from (175 J/m^2) UV- and non-UV-treated mice. Viable cells (2×10^6/ml) were cultured for 3 days in RPMI 1640 supplemented as before. The cell-free supernatants were dialyzed overnight against 50 volumes of RPMI 1640 and then sterilized by Millipore filtration (Millipore Corp., Bedford, Massachusetts). These supernatants were then tested for ETAF activity using the thymocyte proliferation assay [21]. Briefly, 1.5×10^6 C3H/HeJ thymocytes were cultured for 72 h in Costar flat-bottom plates (Costar, Cambridge, Massachusetts) in 200 μl of RPMI 1640 with 10% FCS, penicillin, streptomycin, glutamine, 2-mercaptoethanol (2-ME), 1 μg/ml phytohemagglutinin (PHA), and varying dilutions of the supernatants. Cultures were pulsed with 0.5 μCi [^{3}H]TdR for the final 16 h.

Preparation of Semipurified ETAF

Epidermal cells from non-UV-treated mice were cultured for 3 days as described above. Supernatants were then concentrated 30× using Amicon ultrafiltration cells with PM-10 Diaflo membranes (Amicon Corporation, Lexington, Massachusetts). Three milliliters of the concentrate were then placed on a Sephadex G-50 superfine column using reverse flow. A flow rate of 7 ml/h was used and 3.5-ml fractions were collected. Aliquots of these fractions were diluted 1:4 in RPMI 1640, filter sterilized, and tested for ETAF activity in the thymocyte proliferation assay. When tested in this way, the ETAF preparations used yielded approximately 15,000 cpm [^{3}H]TdR incorporation in the thymocyte assay compared to background of 500 cpm. The active fractions were then obtained (approximate M_r 12,000).

Autologous Epidermal Cell Lymphocyte Reaction

Epidermal cells (2×10^5, 1×10^5, or 0.5×10^5) from UV-treated or non-UV-treated C57Bl/6 mice were treated with 2000 rads x-ray, then incubated with 2×10^5 C57Bl/6 T cells in round-bottom plates (Dynatech). Wells with T cells alone were used to determine background counts. Cells were cultured 5 days at 37°C with 5% CO_2 in humidified air with or without the addition of ETAF, then pulsed with [^{3}H]TdR, harvested, and counted as stated for allogeneic ELR. The results are expressed as the mean cpm [^{3}H]TdR incorporated ± SE of triplicate cultures.

Addition of ETAF to Epidermal Lymphocyte Cultures

Fifty microliters of Sephadex G-50 purified fractions containing ETAF activity were added to 150 μl of cultures (final dilution 1:4) containing responder T cells and epidermal cells from UV-treated mice or non-UV-treated mice. Epidermal lymphocyte cultures were then maintained as before and assessed for alloantigen-presenting ability. In order to determine whether the ETAF preparation used was increasing the stimulatory ability of epidermal cells, ETAF was added to epidermal lymphocyte cultures containing epidermal cells from UV- and non-UV-treated recipients. In addition, ETAF was added to autologous epidermal lymphocyte cultures.

Depletion of Langerhans Cells from Epidermal Cell Suspensions

Since Langerhans cells are the only epidermal cells that bear surface Ia [18], epidermal cell suspensions were depleted of Langerhans cells by treating with anti-Ia antiserum plus complement as described previously [21]. Normal mouse serum plus complement was used as a control. The depleted population contained less than 0.1% Langerhans cells as compared to 1.8% in the control group.

Assessment of Viability after UVR Treatment

In order to determine whether UVR induced selective alterations in Langerhans cell viability, epidermal cells from UV- or non-UV-treated mice were cultured as described above. Viability was assessed by trypan blue dye exclusion on day 0 to day 5. In addition, the percentage of viable cells that were Ia-positive was determined using a modification of the double staining technique of van Rood et al [27], as previously described [23]. Briefly, Langerhans cells were stained with polyspecific Ia antiserum (A.TH anti A.TL) which reacts with Ia of most haplotypes including Ia^d, using a fluorescent 2-step procedure, and cell viability was assessed by the ability to exclude ethidium bromide.

Statistical Analysis

Student's *t*-test was used to assess differences between UV- and non-UV-treated groups. Each experiment was performed at least twice, except for the time course which was performed once. Representative results are presented. *P* values of less than 0.05 were considered significant.

RESULTS

Time Course for T-Cell Stimulation by Allogeneic Epidermal Cells; Epidermal Lymphocyte Reaction

When epidermal cells from BALB/c mice were cocultured with T cells from C57Bl/6 mice, proliferation of T cells (as determined by uptake of [^{3}H]thymidine) peaked on day 5 (Fig 1). This T-cell proliferation was significantly less when epider-

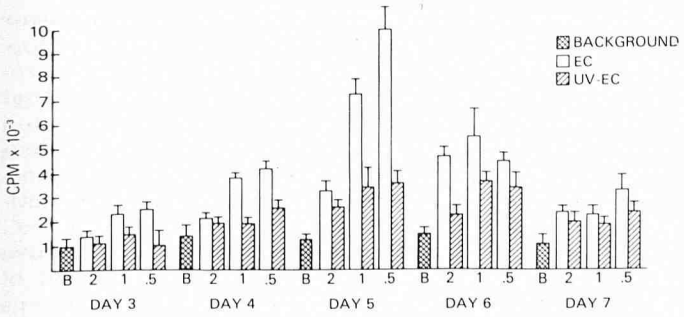

FIG 1. Time course for T-cell stimulation by allogeneic epidermal cells; epidermal lymphocyte reaction (ELR). Varying numbers of epidermal cells (2×10^5, 1×10^5, or 0.5×10^5) from UV-treated or non-UV-treated BALB/c mice were cocultured for 1–14 days with 2×10^5 T cells from C57B1/6 mice. [^{3}H]TdR was added for the last 16 h of culture. *Bars* represent mean cpm [^{3}H]TdR incorporation ± SE. T cells without stimulator cells were used as background counts (*first bar on each day*). From day 0 to day 2 and from day 8 to day 14 no significant stimulation was seen (data not included).

TABLE I. *Assessment of ETAF activity from epidermal cells from UV- or non-UV-irradiated mice*

Source of supernatant[a]	Dilution[b]	cpm [^{3}H]TdR incorporation by C3H/HeJ thymocytes	
		Experiment 1	Experiment 2
UV-EC	1:4	1,856 ± 301[c]	2,228 ± 323[c]
UV-EC	1:8	948 ± 488	942 ± 70
UV-EC	1:16	701 ± 214	584 ± 16
UV-EC	1:32	714 ± 250	518 ± 41
EC	1:4	6,578 ± 986	6,070 ± 736
EC	1:8	3,214 ± 578	2,373 ± 572
EC	1:16	1,008 ± 211	1,097 ± 117
EC	1:32	648 ± 325	734 ± 140
No supernatant added		576 ± 211	689 ± 89

[a] Supernatant prepared by culturing 2×10^6 BALB/c epidermal cells/ml from UV-treated mice (UV-EC) or from non-UV-treated mice (EC) for 3 days. The supernatant was dialyzed and tested for ETAF activity by its ability to augment PHA-induced thymocyte proliferation.
[b] Final dilution of ETAF in the culture.
[c] cpm ± SE.

mal cells from UV-treated mice were used as stimulator cells. Maximum T-cell proliferation was generally seen when 0.5×10^5 or 1×10^5 epidermal cells were used as stimulator cells.

Assessment of ETAF Activity from UV- and Non-UV-Treated Epidermal Cells

When epidermal cells from UV-treated animals were cultured and the supernatant was tested for ETAF activity, there was significantly less activity ($p < .05$) than that detected in supernatants from cells from non-UV-treated mice (Table I).

Reversal of UV-Inhibition of T-Cell Stimulation with ETAF

When fractions of Sephadex G-50 purified ETAF (final dilution 1:4) were added to epidermal cells from UV-treated mice and these cells cocultured with T cells, significant restoration of T-cell stimulation was seen ($p < .01$). Little or no increase in T-cell stimulation was seen when ETAF was added to the cultures containing epidermal cells from non-UV-treated mice (Table II).

Effect of ETAF on Epidermal Lymphocyte Cultures Using Syngeneic Responder and Stimulator Cells

As shown in Table III using a 5-day culture, no significant autologous epidermal lymphocyte reaction was seen with or without the addition of ETAF. There was, however, a significant allogeneic epidermal lymphocyte reaction which was inhibited by UVR and partially restored with ETAF.

Requirement for Epidermal Langerhans Cells in the Induction of Allogeneic T-Cell Stimulation

When BALB/c epidermal cells were treated with anti-Ia antiserum (A.TH anti A.TL) plus complement, and these Langerhans cell-depleted epidermal cells were used as stimulator cells, there was no significant T-cell stimulation. In addition, when ETAF was added to these Langerhans cell-depleted cultures, there was no increase in T-cell proliferation (Table IV). This implies that Langerhans cells are needed for T-cell stimulation and that ETAF alone will not restore stimulation.

Effect of UVR on Epidermal Cell Viability

Table V shows that viability of epidermal cells from UV-treated animals is not significantly different from epidermal

TABLE II. *UV alteration in the ability of epidermal cells to act as stimulator cells for allogeneic T cells: restoration with ETAF*

Stimulator cells[a]	cpm [^{3}H]TdR incorporation by responder T cells	
	Without ETAF	With ETAF[b]
2×10^5 EC (BALB/c)	6,618 ± 727[c]	5,516 ± 675[c]
1×10^5 EC (BALB/c)	9,841 ± 682	8,895 ± 275
0.5×10^5 EC (BALB/c)	7,162 ± 1,197	7,888 ± 596
2×10^5 UV-EC (BALB/c)	3,022 ± 389	4,430 ± 158
1×10^5 UV-EC (BALB/c)	3,334 ± 1,117	6,041 ± 193
0.5×10^5 UV-EC (BALB/c)	3,450 ± 580	6,004 ± 259
		1,050 ± 388
No stimulator cells	1,178 ± 256	1,050 ± 388

[a] Epidermal cells were prepared from non-UV-treated BALB/c mice (EC) or from UV-treated mice (UV-EC).
[b] ETAF prepared from column purified supernatant from cultured BALB/c epidermal cells and added to the cultures to make a final dilution of 1:4.
[c] cpm ± SE [^{3}H]TdR incorporation by T cells from C57Bl/6 mice cocultured 5 days with UV-EC, with EC, or alone.

TABLE III. *Epidermal cells stimulate allogeneic but not syngeneic T cells*

Stimulator cells[a]	cpm [^{3}H]TdR incorporation by responder T cells from C57Bl/6 mice	
	Without ETAF	With ETAF[b]
2×10^5 EC (BALB/c)	4,147 ± 495[c]	4,019 ± 219[c]
1×10^5 EC (BALB/c)	5,235 ± 580	4,718 ± 501
0.5×10^5 EC (BALB/c)	2,821 ± 383	4,145 ± 510
2×10^5 UV-EC (BALB/c)	1,563 ± 721	3,048 ± 309
1×10^5 UV-EC (BALB/c)	1,136 ± 374	3,856 ± 513
0.5×10^5 UV-EC (BALB/c)	1,287 ± 518	2,013 ± 259
2×10^5 EC (B6)	1,102 ± 484	918 ± 79
1×10^5 EC (B6)	1,236 ± 532	1,121 ± 721
0.5×10^5 EC (B6)	908 ± 211	856 ± 256
2×10^5 UV-EC (B6)	899 ± 194	799 ± 413
1×10^5 UV-EC (B6)	1,118 ± 316	1,209 ± 425
0.5×10^5 UV-EC (B6)	868 ± 306	999 ± 187

[a] Epidermal cells were prepared from nonirradiated BALB/c mice [EC(BALB/c)], non-UV-irradiated C57Bl/6 mice [EC(B6)], or from UV-irradiated BALB/c or C57Bl/6 mice [UV-EC(BALB/c) and UV-EC(B6)].
[b] ETAF prepared from column purified supernatant from cultured BALB/c epidermal cells and added to the cultures to make a final dilution of 1:4.
[c] cpm ± SE [^{3}H]TdR incorporation by T cells from C57Bl/6 mice cocultured 5 days with UV-EC, with EC, or alone.

TABLE IV. *Requirement for epidermal Langerhans cells for stimulation of allogeneic T cells*

Stimulator cells[a]	cpm [^{3}H]TdR incorporation by responder T cells	
	Without ETAF	With ETAF[b]
2×10^5 EC (NMS + C′)	3,702 + 286[c]	4,078 + 595[c]
1×10^5 EC (NMS + C′)	6,908 + 1,666	7,001 + 286
0.5×10^5 EC (NMS + C′)	7,751 + 329	6,543 + 711
2×10^5 EC (αIa + C′)	1,714 + 500	1,528 + 758
1×10^5 EC (αIa + C′)	1,226 + 53	1,311 + 256
0.5×10^5 EC (αIa + C′)	2,130 + 714	2,005 + 819
None	1,178 + 256	1,050 + 388

[a] Stimulator cells were BALB/c epidermal cells treated with normal mouse serum plus complement EC (NMS + C′) or anti-Ia antiserum plus complement EC (αIa + C′).

[b] ETAF prepared from column purified supernatant from cultured BALB/c epidermal cells and added to the cultures to make a final dilution of 1:4.

[c] cpm + SE [^{3}H]TdR incorporation by T cells from C57Bl/6 mice cocultured for 5 days with αIa plus complement-treated BALB/c epidermal cells. The above groups were cultured with or without a 25% (final concentration) solution of column purified ETAF.

TABLE V. *Viability of cultured epidermal cells and Langerhans cells after UV irradiation*

	Percentage viable epidermal cells/percentage viable cells that were Ia positive[a]					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
EC	89/1.7	64/1.9	34/1.0	26/0.6	24/0.2	19/0.1
UV-EC	85/1.8	57/1.5	39/1.0	31/0.7	17/0.3	15/0.09

[a] Epidermal cells from UV-treated or non-UV-treated mice were cultured for 5 days. Percentage of viable epidermal cells and the percentage of viable epidermal cells that were Ia positive were determined daily. Overall viability was assessed by trypan blue dye exclusion and the percentage of viable cells that were Ia positive (Langerhans cells) was assessed by simultaneous fluorescent Ia staining and ethidium bromide staining; approximately 300 cells were counted in each group.

cells from non-UV-treated mice. In addition, by using a double fluorescent staining technique with anti-Ia antiserum and ethidium bromide to specifically look at viable Langerhans cells (i.e., Ia-stained cells that excluded ethidium bromide), it was found that there was no difference in Langerhans cell viability between the 2 groups. In addition, there was no alteration in surface Ia as detected by indirect immunofluorescence.

DISCUSSION

Ia-bearing macrophages are considered to be the major stimulatory cells in the mixed lymphocyte reaction [1–7]. Steinman and Nussenzweig have shown that certain murine splenic dendritic cells are also capable of stimulating a mixed lymphocyte reaction [28]. Human epidermal cells have also been shown to cause stimulation of allogeneic lymphocytes [29,30]. Using murine epidermal cells, we have shown that epidermal cells are capable of acting as stimulator cells for allogeneic T cells (epidermal lymphocyte reaction), that the stimulatory cell is the Langerhans cell, and that UVR is capable of abrogating this reaction. The latter finding is in keeping with in vitro studies of Lafferty and Woolnough [12], Lindahl-Kiesling and Safwenberg [31], and Rollinghoff and Wagner [32], who have shown that UVR treatment of stimulator cells alters alloantigen stimulation.

UVR has profound effects on the immune system both in vivo and in vitro. UV-treated mice have a reduced capacity to reject UV-induced tumors [33,34] and do not show a normal graft-vs.-host reaction when allogeneic lymphocytes are injected into their foot pads [35]. UVR is also capable of suppressing allergic contact sensitization [26,36,37]. The mechanism behind these in vitro or in vivo UVR alterations in immune function has been the subject of considerable investigation. Some studies have suggested that UVR may affect IL-1 production [12,17,38]. Lafferty and Woolnough [12] postulated that the UV-induced defect in alloantigen presentation was related to the inability of UV-treated cells to provide a soluble "costimulator" function, termed "second signal." It has been postulated that interleukin 1 plays an integral role in T-cell activation responses and may provide this second signal [16].

The data presented here are in keeping with the above findings. In particular, we have shown that UV treatment of epidermal cells reduces their ability to present alloantigens without affecting cell viability. Furthermore, this defect is associated with a decrease in the production of the soluble epidermal cell product ETAF. In addition, this UV-induced defect can be partially reversed with the addition of exogenous ETAF. It is not clear whether UVR affects ETAF alone or whether there is also a UV-induced alteration of Langerhans cells whereby their stimulatory ability is affected. It is possible that Ia antigens on the Langerhans cells are functionally altered and yet still detectable by immunofluorescence staining. We would favor the hypothesis that UVR, in addition to altering ETAF production, alters Langerhans cell alloantigen-presenting function to some degree. The studies of Germain [17], Weinberger et al [38], and the recent studies of Jakway et al [39] using in vitro UVR would support the hypothesis that UVR alters antigen processing as well as IL-1 production. Germain showed that if antigen-presenting cells were UV-treated before pulsing with antigen, these cells failed to support antigen-specific T-cell proliferation even in the presence of PMA, a potent stimulator of IL-1 production. In a similar line of investigation, Weinberger et al [38] demonstrated that when splenic adherent cells were UV-treated prior to pulsing with H-2K^k antigen, they were incapable of inducing cytotoxic T cells, but if they were UV-treated after pulsing with H-2K^k antigen, cytotoxic T cells could be restored in the presence of PMA. Similar findings were shown by Jakway et al [39], using an in vitro guinea pig antigen presentation system and guinea pig IL-1. They showed that antigen presentation could not be restored with IL-1 if macrophages were UV-treated prior to pulsing with antigen, but antigen presentation could be partially restored with IL-1 if macrophages were treated after pulsing with antigen. These findings suggest that UVR may interfere with antigen processing as well as IL-1 production. Our system is, however, not directly analogous to the above system since we are irradiating the mice in vivo, then preparing epidermal cells from these animals, and using these epidermal cells as stimulator cells for allogeneic T cells. Presumably, our pretreatment with UVR only partially inactivates the stimulator cells and, therefore, restoration is possible. When stimulator cells are inactivated with anti-Ia antiserum and complement, no restoration is achieved.

UV alteration of alloantigen presentation by epidermal cells may provide a unique opportunity to dissect out the various signals necessary for alloantigen presentation. In the conventional systems of antigen presentation by macrophages, macrophages are the source of both signal 1 and signal 2. Like macrophages, Langerhans cells are the source of processed antigen (signal 1), but it is unknown whether Langerhans cells produce any ETAF (signal 2) [21]. One source of the second signal is clearly the keratinocyte [21,22]. Therefore, within the epidermis, one cell type, the Langerhans cell, provides the first signal, i.e., the formation of the Ia-alloantigen complex, but may not provide the nonspecific second signal. The nonspecific second signal can be provided by the keratinocyte, which is incapable of antigen presentation. The epidermis thus has 2 distinct cell populations which may provide separate but necessary signals for antigen presentation. Keratinocyte cell lines are available, and hopefully in the near future Langerhans cell lines may be derived. These distinctive cell populations thus may provide the potential to dissect out the various signals necessary for antigen presentation.

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